Journal of Peptide Science
J. Peptide Sci. **5**: 547–554 (1999)

Preferred Solution Conformation of Peptides Rich in the Lipophilic, Chiral, C^{α}-Methylated α-Amino Acid (αMe)Aoc

CRISTINA PEGGION[a], FERNANDO FORMAGGIO[a], MARCO CRISMA[a], CLAUDIO TONIOLO[a,*], BERNARD KAPTEIN[b], QUIRINUS B. BROXTERMAN[b] and JOHAN KAMPHUIS[c]

[a] Biopolymer Research Centre, CNR, Department of Organic Chemistry, University of Padova, 35131 Padova, Italy
[b] DSM Research, Organic Chemistry and Biotechnology Section, PO Box 18, 6160 MD Geleen, Netherlands
[c] DSM Specialty Intermediates, PO Box 5489, 6130 PL Sittard, Netherlands

Received 14 June 1999
Accepted 22 June 1999

Abstract: The lipophilic, chiral, C^{α}-methylated α-amino acid L-(αMe)Aoc (2-methyl-2-amino-octanoic acid) was prepared using a chemo-enzymatic approach. Two series of terminally protected model peptides, from dimer through to hexamer, containing L-(αMe)Aoc in combination with either Gly or Aib, were synthesized by solution methods and were fully characterized. A solution conformational analysis, based on FT-IR absorption, ^{1}H-NMR and circular dichroism (CD) techniques, was performed with the aim at determining the preferred conformation of this novel amino acid and the relationship between chirality at its α-carbon atom and screw sense of the helix that is formed. The results obtained strongly support the view that L-(αMe)Aoc favours the formation of the *right*-handed 3_{10}-helical conformation. Copyright © 1999 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: α-aminoisobutyric acid; 3_{10}-helix; hydrophobicity; lipophilic side-chain; C^{α}-methyl, C^{α}-*n*-hexylglycine; peptide conformation; peptide synthesis; spectroscopy

INTRODUCTION

Interest in chiral, C^{α}-methylated, α-amino acids stems from their capability to induce significant restraints on peptide backbone conformational freedom [1,2]. In particular, these building blocks have gained importance in the design of analogues of bioactive compounds [3–11]. The conformational propensity of C^{α}-methylated analogues of protein amino acids, such as (αMe)Val [2,12,13], (αMe)Leu [2], (αMe)Phe [2] and (αMe)Trp [14], when introduced in peptide sequences, have been extensively investigated.

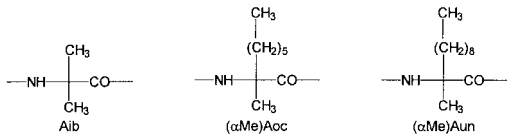
The possibility of combining the conformational rigidity that C^{α}-methylation induces in the peptide backbone with the specific properties of the amino acid side-chains has attracted the attention of chemists towards the production of an increasing number of compounds of this class. In particular, as lipophilicity plays an important role in improving peptide drug bioavailability, C^{α}-*un*methylated lipoamino acids have been frequently introduced into peptides to increase their overall hydrophobicity and to impart to them a membrane-like character [15–20]. In this way, the passage of poorly absorbed drugs across biological membranes is increasingly facilitated. Interestingly, the combination of the atypical nature of a lipoamino acid with the even more atypical nature of a C^{α}-methylated amino

Abbreviations: Aib, α-aminoisobutyric acid or $C^{\alpha,\alpha}$-dimethylglycine; (αMe)Aoc, C^{α}-methyl, C^{α}-*n*-hexylglycine or 2-methyl-2-amino-octanoic acid; (αMe)Aun, C^{α}-methyl, C^{α}-*n*-nonylglycine or 2-methyl-2-amino-undecanoic acid; DMSO, dimethylsulphoxide; EDC, *N*-ethyl, *N*′-[3-(dimethylamino)propyl]carbodiimide; HOAt, 1-hydroxy-7-azabenzotriazole; NMM, *N*-methylmorpholine; Z, benzyloxycarbonyl; MeCN, acetonitrile; MeOH, methanol; TEMPO, 2,2,6,6-tetramethylpiperidinyl-1-oxy; TFE, 2,2,2-trifluoroethanol.

* Correspondence to: Biopolymer Research Centre, CNR, Department of Organic Chemistry, University of Padova, via Marzolo 1, 35131 Padova, Italy. E-mail: biop02@chor.unipd.it

CCC 1075–2617/99/120547-08$17.50

acid might also contribute to imparting a high degree of resistance to proteolytic enzymes, known to be responsible for the chemical instability of peptide drugs. In this context, recently, the highly lipophilic, chiral, C^{α}-methylated α-amino acid L-(αMe)Aun has been introduced in an analogue of the membrane-active lipopeptaibol antibiotic trichogin GA IV, where it successfully replaced the naturally occurring *N*-terminal *n*-octanoyl chain, essential for activity [21].



As no information is available to date on the conformational propensity of lipophilic, chiral, C^{α}-methylated α-amino acids, this paper presents the synthesis, characterization and conformational analysis [by FT-IR absorption, NMR and circular dichroism (CD) techniques] of a number of model peptides containing L-(αMe)Aoc in combination with Aib or Gly.

MATERIALS AND METHODS

Peptide Synthesis

Melting points were determined using a Leitz (Wetzlar, Germany) model Laborlux 12 apparatus and were not corrected. Optical rotations were measured using a Perkin–Elmer (Norwalk, CT) model 241 polarimeter equipped with a Haake (Karlsruhe, Germany) model D thermostat. Thin layer chromatography (TLC) was performed on Merck (Darmstadt, Germany) Kieselgel $60/F_{254}$ precoated plates. The chromatograms were developed by quenching of UV fluorescence, chlorine–starch–potassium iodide or ninhydrin chromatic reaction as appropriate.

Infrared Absorption

The solid state infrared absorption spectra (KBr disk technique) were recorded with a Perkin–Elmer model 580 B spectrophotometer equipped with a Perkin–Elmer model 3600 IR data station. The solution IR absorption spectra were recorded using a Perkin–Elmer model 1720 X FT-IR spectrophotometer, nitrogen-flushed, equipped with a sample shuttle device, at 2 cm^{-1} nominal resolution, averaging 100 scans. Solvent (baseline) spectra were obtained under the same conditions. Cells with path lengths of 0.1, 1.0 and 10 mm (with CaF_2 windows) were used. Spectrograde deuterochloroform (99.8% d) was purchased from Fluka (Buchs, Switzerland).

Circular Dichroism

The CD spectra were obtained on a Jasco (Tokyo, Japan) model J-715 dichrograph. Cylindrical fused quartz cells of 1.0 and 0.2 mm path lengths were used. The values are expressed in terms of $([\theta]_T$, the total molar ellipticity (deg cm^2 $dmol^{-1}$). Spectrograde MeOH and TFE (Acros Organics, Geel, Belgium) were used as solvents.

^{1}H-Nuclear Magnetic Resonance

The ^{1}H-NMR spectra were recorded with a Bruker (Karlsruhe, Germany) model AM 400 spectrometer. Measurements were carried out in deuterochloroform (99.96% d; Aldrich, Milwaukee, WI) and deuterated dimethylsulphoxide (99.96% d_6; Acros Organics, Geel, Belgium) with tetramethylsilane as the internal standard. The free radical TEMPO was purchased from Sigma (St. Louis, MO).

RESULTS AND DISCUSSION

Peptide Synthesis

For the large-scale production of the enantiomerically pure L-(αMe)Aoc, an economically attractive and generally applicable chemo-enzymatic synthesis, developed by DSM Research [22–24] a few years ago, was exploited. It involves a combination of partial Strecker synthesis for the preparation of the racemic α-amino acid amide followed by the use of a broadly specific amino acid amidase from *Mycobacterium neoaurum* to achieve optical resolution. The synthesis of racemic (αMe)Aoc and the gas chromatographic separation of selected diastereomeric esters at the analytical level have already been published [25]. The Z-protected derivative was prepared by reacting the free amino acid with *N*-(benzyloxycarbonyl)-succinimide in MeCN in the presence of the lipophilic base tetramethylammonium hydroxide [26]. Gly and Aib *tert*-butyl esters were obtained by esterification of the corresponding Z-protected amino acid with isobutylene in the presence of a catalytic amount of sulphuric acid [27]. Peptide bond formation was achieved by the EDC/HOAt method [28] in CH_2Cl_2 in the presence of NMM. Using this approach, formation of the sterically hindered (αMe)Aoc-Aib and Aib-(αMe)Aoc peptide

bonds was obtained in moderately good yields (55–70%). Removal of the Z *N*-protecting group was performed by catalytic hydrogenation. The N^{α}-blocked hexapeptide Ac-Aib-L-(αMe)Aoc-Aib-Aib-L-(αMe)Aoc-Aib-O*t*Bu was prepared by treatment of H-Aib-L-(αMe)Aoc-Aib-Aib-L-(αMe)Aoc-Aib-O*t*Bu with ten equivalents of acetic anhydride in CH_2Cl_2. The N^{α}-protected peptide free acids were prepared from the corresponding *tert*-butyl esters by treatment with diluted trifluoroacetic acid. The physical and analytical properties of the (αMe)Aoc derivatives and peptides are listed in Table 1. All compounds were also characterized by ^{1}H-NMR.

Solution Conformational Analysis

The solution conformational preferences of the (αMe)Aoc/Aib and (αMe)Aoc/Gly model peptides were determined by FT-IR absorption, ^{1}H-NMR and CD techniques.

Figure 1 shows the FT-IR absorption spectra (N–H stretching region) of the (αMe)Aoc/Aib series from di to hexapeptide in $CDCl_3$ solution. The curves of the higher homologues are characterized by two bands at about 3430 cm^{-1}, assigned to the free (solvated) NH groups, and at 3380–3340 cm^{-1}, assigned to H-bonded NH groups [29]. The intensity of the low-frequency band, relative to the high-frequency band, considerably increases as the main chain length increases. Concomitantly, the absorption maximum markedly shifts to lower wavenumbers. Figure 2 illustrates the FT-IR absorption spectra of the (αMe)Aoc/Gly series from di to pentapeptide in the same spectral region. A behaviour similar to that exhibited by the (αMe)Aoc/Aib series, albeit less pronounced, is observed. In the (αMe)Aoc/Gly di, tri and tetrapeptides, a significant amount of unordered conformation is still present, together with a modest amount of H-bonded conformations. Only in the pentapeptide, with two (αMe)Aoc residues, a significant shift to a lower frequency and a remarkable increase in intensity of the absorption band of the H-bonded NH groups, indicative of the onset of a stable helical structure, are seen. Upon changing the concentration (in the 10–0.1 mM range) all model peptides display only minor variations in the spectra (results not shown). The present FT-IR absorption investigation provided convincing evidence that the (αMe)Aoc/Aib peptides are highly folded in a helical conformation, whereas a significant flexibility seems to be characteristic of the lower members of the (αMe)Aoc/Gly series.

To get more detailed information on the preferred conformation of the (αMe)Aoc-rich peptides, a ^{1}H-NMR study in a $CDCl_3$ solution at 1.0 mM concentration was performed, where self-association is

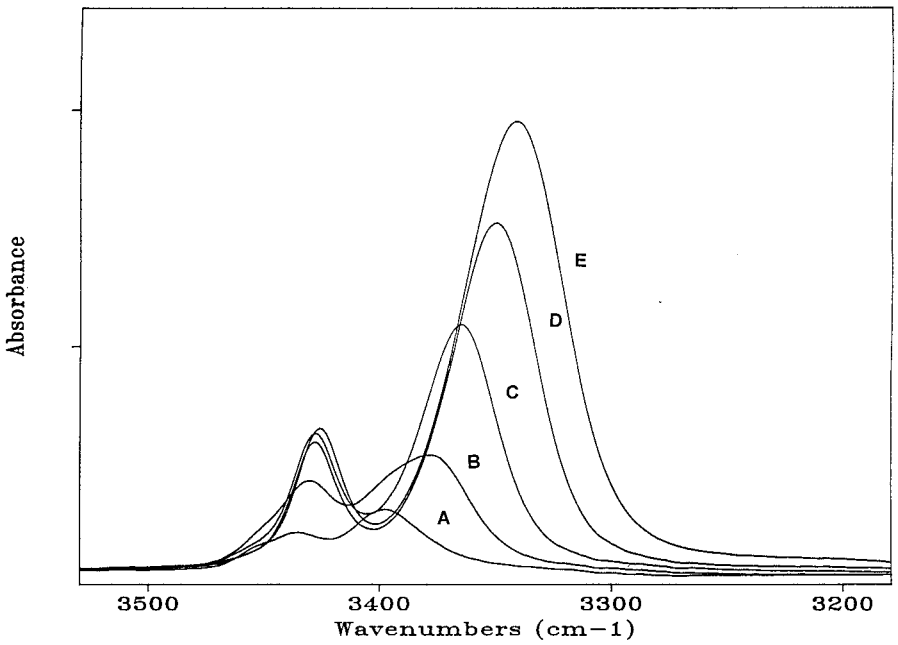


Figure 1 FT-IR absorption spectra in the 3500–3200 cm^{-1} region of Z-L-(αMe)Aoc-Aib-O*t*Bu (A), Z-Aib-L-(αMe)Aoc-Aib-O*t*Bu (B), Z-Aib-Aib-L-(αMe)Aoc-Aib-O*t*Bu (C), Z-L-(αMe)Aoc-Aib-Aib-L-(αMe)Aoc-Aib-O*t*Bu (D), and Z-Aib-L-(αMe)Aoc-Aib-Aib-L-(αMe)Aoc-Aib-O*t*Bu (E) in $CDCl_3$ solution. Peptide concentration: 1.0 mM.

Table 1 Physical and Analytical Properties for the (αMe)Aoc Derivatives and Peptides

Compound	Yield	Melting point	Recrystallization solvent[a]	$[\alpha]_D^{20}$	TLC[c]			IR[d]
	(%)	(°C)		(°)[b]	R_{F_I}	$R_{F_{II}}$	$R_{F_{III}}$	(cm^{-1})
Z-L-(αMe)Aoc-OH	90	Oil	–	3.6	0.70	0.90	0.30	3414, 3333, 1706, 1585
Z-L-(αMe)Aoc-Aib-O*t*Bu	70	89–91	EtOAc/PE	2.0	0.90	0.95	0.60	3404, 3294, 1722, 1658, 1537
Z-L-(αMe)Aoc-Aib-OH	96	128–130	Et_2O/PE	1.1	0.45	0.95	0.20	3427, 3293, 1719, 1703, 1655, 1590, 1529
Z-Aib-L-(αMe)Aoc-Aib-O*t*Bu	55	Oil	–	−6.0	0.90	0.95	0.40	3432, 3350, 1731, 1704, 1526
Z-Aib-L-(αMe)Aoc-Aib-OH	70	141–143	Et_2O/PE	−1.8	0.40	0.95	0.20	3314, 1735, 1698, 1659, 1526
Z-Aib-Aib-L-(αMe)Aoc-Aib-O*t*Bu	65	158–160	EtOAc/PE	−17.0	0.80	0.95	0.25	3425, 3335, 1728, 1683, 1668, 1534
Z-Aib-Aib-L-(αMe)Aoc-Aib-OH	75	Oil	–	−5.5	0.35	0.95	0.15	3314, 1703, 1660, 1529
Z-L-(αMe)Aoc-Aib-Aib-L-(αMe)Aoc-Aib-O*t*Bu	60	205–206	EtOAc/PE	−5.6	0.90	0.95	0.25	3417, 3322, 1724, 1699, 1663, 1532
Z-Aib-L-(αMe)Aoc-Aib-Aib-L-(αMe)-Aoc-Aib-O*t*Bu	61	146–147	EtOAc/PE	−12.0	0.80	0.95	0.35	3427, 3322, 1730, 1701, 1661, 1530
Ac-Aib-L-(αMe)Aoc-Aib-Aib-L-(αMe)-Aoc-Aib-O*t*Bu	70	85–86	CH_2Cl_2/PE	−3.5	0.40	0.90	0.15	3307, 1730, 1659, 1535
Z-L-(αMe)Aoc-Gly-O*t*Bu	97	Oil	–	2.6	0.90	0.90	0.65	3374, 1726, 1660, 1584
Z-Gly-L-(αMe)Aoc-Gly-O*t*Bu	80	114–115	EtOAc/PE	1.6	0.90	0.95	0.30	3353, 3275, 1730, 1701, 1677, 1639, 1552
Z-Gly-Gly-L-(αMe)Aoc-Gly-O*t*Bu	87	125–126	EtOAc/PE	−0.8	0.65	0.95	0.20	3339, 1745, 1715, 1663, 1524
Z-L-(αMe)Aoc -Gly-Gly-L-(αMe)Aoc-Gly-O*t*Bu	88	59–60	Et_2O/PE	−1.9	0.65	0.95	0.20	3315, 1744, 1660, 1533

[a] EtOAc, ethyl acetate; PE petroleum ether; Et_2O, diethyl ether.
[b] $c = 0.5$, MeOH.
[c] R_{F_I} = $CHCl_3$/EtOH, 9:1; $R_{F_{II}}$ = 1-BuOH/AcOH/H_2O, 3:1:1; $R_{F_{III}}$ = toluene/EtOH, 7:1.
[d] The IR absorption spectra were obtained in KBr pellets (only bands in the 3500–3200 and 1800–1500 cm^{-1} regions are reported).

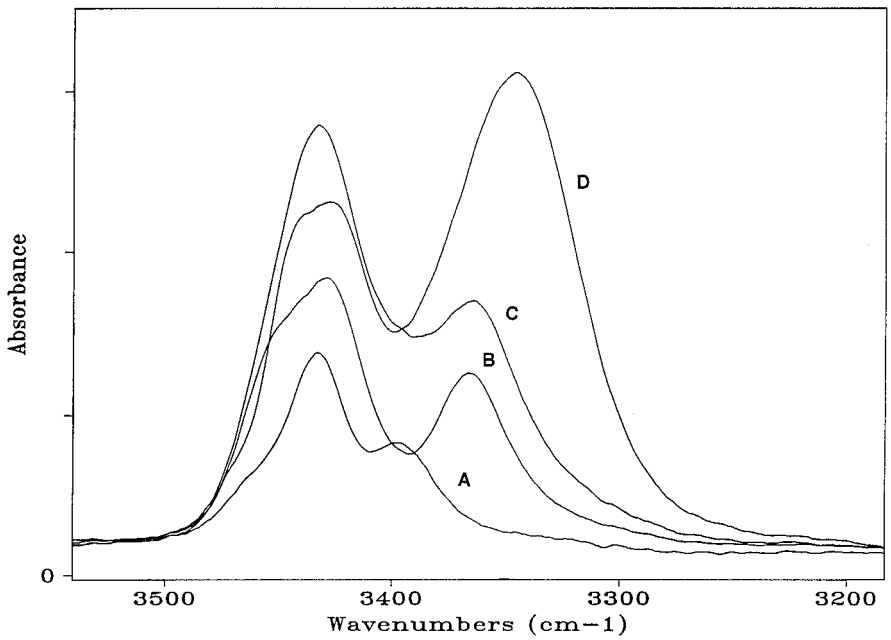


Figure 2 FT-IR absorption spectra in the 3500–3200 cm^{-1} region of Z-L-(αMe)Aoc-Gly-O*t*Bu (A), Z-Gly-L-(αMe)Aoc-Gly-O*t*Bu (B), Z-Gly-Gly-L-(αMe)Aoc-Gly-O*t*Bu (C), and Z-L-(αMe)Aoc-Gly-Gly-L-(αMe)Aoc-Gly-O*t*Bu (D) in $CDCl_3$ solution. Peptide concentration: 1.0 mM.

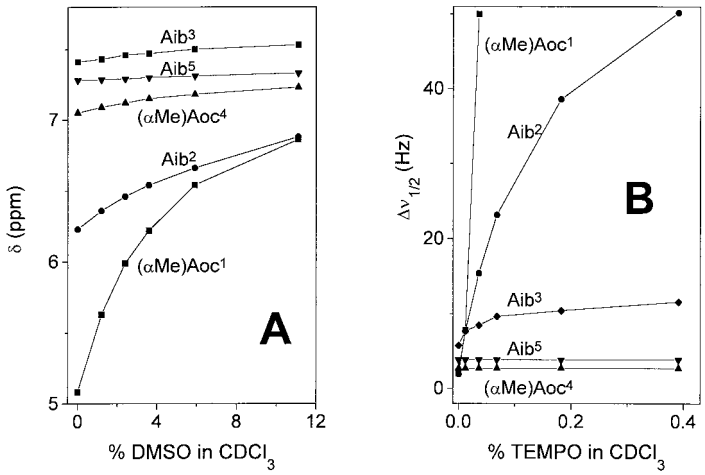


Figure 3 ^{1}H-NMR titration of Z-L-(αMe)Aoc-Aib-Aib-L-(αMe)Aoc-Aib-O*t*Bu: (A) plot of NH chemical shifts as a function of increasing percentages of DMSO added to the $CDCl_3$ solution (v/v); (B) plot of bandwidth of the NH signals as a function of increasing percentages of TEMPO (w/v) in $CDCl_3$. Peptide concentration: 1.0 mM.

absent. The delineation of inaccessible (intramolecularly H-bonded) NH groups was carried out by analysing the behaviour of the NH resonances upon addition of two perturbing agents. In particular, (i) the solvent dependence of NH chemical shifts was examined by adding increasing amounts of the strong H-bonding acceptor solvent DMSO [30,31] to the $CDCl_3$ solution, and (ii) the line broadening of NH resonances induced by adding the free radical TEMPO was also examined [32].

Figure 3 illustrates the behaviour of the NH resonances of the (αMe)Aoc/Aib pentapeptide upon addition of DMSO and TEMPO. Figure 4 shows the behaviour of the related (αMe)Aoc/Gly pentapeptide under the same conditions. All -CONH- proton resonances were assigned by means of 2D ROESY

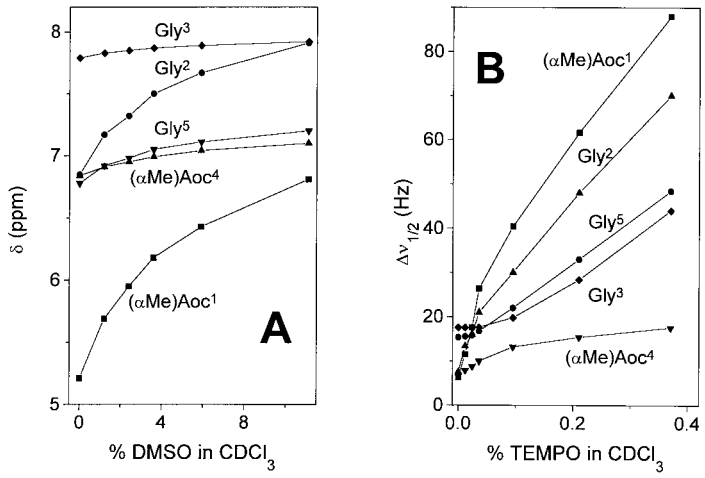


Figure 4 ^{1}H-NMR titration of Z-L-(αMe)Aoc-Gly-Gly-L-(αMe)Aoc-Gly-O*t*Bu: (A) plot of NH chemical shifts as a function of increasing percentages of DMSO added to the $CDCl_3$ solution (v/v); (B) plot of bandwidth of the NH signals as a function of increasing percentages of TEMPO (w/v) in $CDCl_3$. Peptide concentration: 1.0 mM.

experiments. In each peptide two classes of NH protons were found. Class (i) [N(1)H and N(2)H protons] includes protons whose chemical shifts are remarkably sensitive to the addition of DMSO and whose resonances broaden significantly upon addition of the paramagnetic perturbing agent TEMPO. Class (ii) [N(3)H, N(4)H and N(5)H protons] includes those displaying a behaviour characteristic of shielded protons (relative insensitivity of the chemical shift to solvent composition and of the line width to the presence of TEMPO).

To summarize, these ^{1}H-NMR results allow us to conclude that, in a $CDCl_3$ solution, the N(3)–N(5)H protons of the two pentapeptides are almost inaccessible to perturbing agents and are therefore most probably intramolecularly H-bonded. Therefore, it is reasonable that the most populated conformation adopted in the $CDCl_3$ solution by the terminally protected (αMe)Aoc-rich pentapeptides would be the 3_{10}-helix, where only the two *N*-terminal NH protons do not participate in the intramolecular H-bonding scheme. However, the N(3)H and N(5)H protons of the pentapeptide *Z*-(αMe)Aoc-$(Gly)_2$-(αMe)Aoc-Gly-O*t*Bu exhibit a higher sensitivity to the addition of TEMPO as compared with that of the corresponding protons of *Z*-(αMe)Aoc-$(Aib)_2$-(αMe)Aoc-Aib-O*t*Bu. In agreement with the FT-IR absorption results, this observation points to a higher degree of conformational flexibility in the (αMe)Aoc/Gly peptides.

The hexapeptide Ac-Aib-L-(αMe)Aoc-$(Aib)_2$-L-(αMe)Aoc-Aib-O*t*Bu, lacking any potentially disturbing chromophoric group at the *N*-terminus, was synthesized with the aim of determining by CD the relationship between chirality at the α-carbon atom of this new α-amino acid and the screw sense of the helix that is formed. The CD spectrum of this (L,L)-peptide in MeOH solution (Figure 5), displaying a negative maximum centred at 203 nm and a weak, negative shoulder at about 220 nm, strongly resembles the dichroic pattern canonical for a *right*-handed 3_{10}-helix [33]. This type of helical structure appears to be rather stable, as no relevant changes are observed by changing the solvent polarity. From

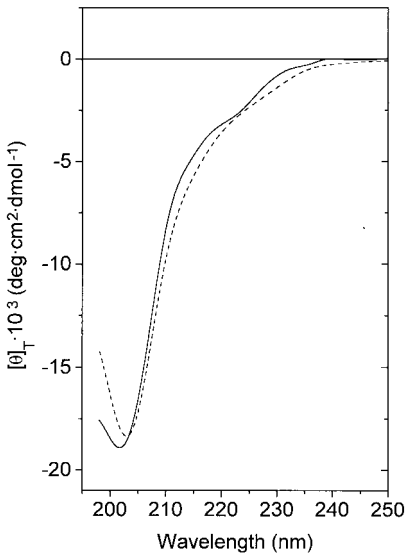


Figure 5 CD spectra of Ac-Aib-L-(αMe)Aoc-Aib-Aib-L-(αMe)Aoc-Aib-O*t*Bu in MeOH (solid line) and TFE (dashed line) solutions. Peptide concentration: 0.5 mM.

this study the additional information can be extracted that the screw sense of the helix formed by this C^{α}-methylated α-amino acid is the same as that characteristic of protein amino acids (an L-amino acid gives a *right*-handed helix).

CONCLUSIONS

The experimental results reported in this work have contributed to elucidate the conformational preferences of the lipophilic, chiral, C^{α}-methylated α-amino acid (αMe)Aoc. The FT-IR absorption, CD and NMR analyses agree with a highly populated 3_{10}-helical motif for the longer model peptides in solution. Moreover, we have been able to show that L-(αMe)Aoc induces the formation of a *right*-handed helical structure. Finally, a study of the dependence of the preferred conformation in chloroform solution on peptide concentration did not reveal any aggregation phenomenon driven by the side-chain of this residue. The authors foresee a bright future for the new class of strongly helicogenic and highly lipophilic α-amino acids, of which (αMe)Aoc and (αMe)Aun [21] are significant members.

REFERENCES

1. Toniolo C, Benedetti E. Structures of polypeptides from α-amino acids disubstituted at the α-carbon. *Macromolecules* 1991; **24**: 4004–4009.
2. Toniolo C, Crisma M, Formaggio F, Valle G, Cavicchioni G, Précigoux G, Aubry A, Kamphuis J. Structures of peptides from α-amino acids methylated at the α-carbon. *Biopolymers* 1993; **33**: 1061–1072.
3. Hruby VJ, Al-Obeidi F, Kazmierski W. Emerging approaches in the molecular design of receptor-selective peptide ligands: conformational, topographical and dynamic considerations. *Biochem. J.* 1990; **268**: 249–262.
4. Karle IL, Balaram P. Structural characteristics of α-helical peptide molecules containing Aib residues. *Biochemistry* 1990; **29**: 6747–6756.
5. Toniolo C. Conformationally restricted peptides through short-range cyclizations. *Int. J. Pept. Protein Res.* 1990; **35**: 287–300.
6. Balaram P. Non-standard amino acids in peptide design and protein engineering. *Curr. Opin. Struct. Biol.* 1992; **2**: 845–851.
7. Giannis A, Kolter T. Peptidomimetics for receptor ligands. Discovery, development, and medical perspectives. *Angew. Chem. Int. Ed. Engl.* 1993; **32**: 1244–1267.
8. Toniolo C. $C^{\alpha,\alpha}$-symmetrically disubstituted glycines: useful building blocks in the design of conformationally restricted peptides. *Janssen Chim. Acta* 1993; **11**: 10–16.
9. Liskamp RMJ. Conformationally restricted amino acids and dipeptides, (non)peptidomimetics and secondary structure mimetics. *Recl. Trav. Chim. Pays-Bas* 1994; **113**: 1–19.
10. Gante J. Peptidomimetics: taylored enzyme inhibitors. *Angew. Chem. Int. Ed. Engl.* 1994; **33**: 1699–1720.
11. Adang AEP, Hermkens PHH, Linders JTM, Ottenheijm HCJ, van Staveren CJ. Case histories of peptidomimetics: progression from peptides to drugs. *Recl. Trav. Chim. Pays-Bas* 1994; **113**: 63–78.
12. Yoder G, Polese A, Silva RAGD, Formaggio F, Crisma M, Broxterman QB, Kamphuis J, Toniolo C, Keiderling TA. Conformational characterization of terminally blocked L-(αMe)Val homopeptides, using vibrational and electronic circular dichroism. 3_{10}-Helical stabilization by peptide–peptide interaction. *J. Am. Chem. Soc.* 1997; **117**: 10278–10285.
13. Polese A, Formaggio F, Crisma M, Valle G, Toniolo G, Bonora GM, Broxterman QB, Kamphuis J. Peptide helices as rigid molecular rulers. A conformational study of isotactic homopeptides from α-methyl-α-isopropylglycine, $[L\text{-}(\alpha Me)Val]_n$. *Chem. Eur. J.* 1996; **2**: 1104–1111.
14. Formaggio F, Toniolo C, Crisma M, Valle G, Kaptein B, Schoemaker HE, Kamphuis J, Di Blasio B, Maglio O, Fattorusso R, Benedetti E, Santini A. The preferred solid-state conformation of (αMe)Trp peptides. *Int. J. Pept. Protein Res.* 1995; **45**: 70–77.
15. Gibbons WA, Hughes RA, Charalambous M, Christodoulou M, Szeto A, Aulabaugh A, Mascagni P, Toth I. Synthesis, resolution and structural elucidation of lipidic amino acids and their homo- and hetero-oligomers. *Liebigs Ann. Chem.* 1990; 1175–1183.
16. Kokotos G, Padron JM, Noula C, Gibbons WA, Martin VS. A general approach to the enantiomeric synthesis of lipidic α-amino acids, peptides and vicinal amino alcohols. *Tetrahedron: Asymm.* 1996; **7**: 857–866.
17. Kellam B, Drouillat B, Dekany G, Starr MS, Toth I. Synthesis and in vitro evaluation of lipoamino acid, and carbohydrate-modified enkephalins as potential antinociceptive agents. *Int. J. Pharm.* 1998; **161**: 55–64.
18. Koppitz M, Huenges M, Gratias R, Kessler H, Goodman SL, Jonczyk A. Synthesis of unnatural lipophilic *N*-(9H-fluoren-9-ylmethoxy)carbonyl-substituted α-amino acids and their incorporation into cyclic RGD-peptides. A structure–activity study. *Helv. Chim. Acta* 1997; **80**: 1280–1300.
19. Koppitz M, Spellig T, Kahmann R, Kessler H. Lipoconjugates. Structure–activity studies for pheromone analogues of *Ustilago maydis* with varied lipophilicity. *Int. J. Pept. Protein Res.* 1996; **48**: 377–390.

20. Kazmierski WM, Hazen RJ, Aulabaugh A, StClair MH. Inhibitors of human immunodeficiency virus type 1 derived from gp41 transmembrane protein. Structure–activity studies. *J. Med. Chem.* 1996; **39**: 2681–2689.
21. Locardi E, Mammi S, Peggion E, Monaco V, Formaggio F, Crisma M, Toniolo C, Bodo B, Rebuffat S, Kamphuis J, Broxterman QB. Conformation and membrane activity of an analogue of the peptaibol antibiotic trichogin GA IV with a lipophilic amino acid at the *N*-terminus. *J. Pept. Sci.* 1998; **4**: 389–399.
22. Kruizinga WH, Bolster J, Kellogg RM, Kamphuis J, Boesten WHJ, Meijer EM, Schoemaker HE. Synthesis of optically pure α-alkylated α-amino acids and a single-step method for enantiomeric excess determination. *J. Org. Chem.* 1988; **53**: 1826–1827.
23. Elferink VHM, Breitgoff D, Kloosterman M, Kamphuis J, van den Tweel WJJ, Meijer EM. Industrial developments in biocatalysis. *Recl. Trav. Chim. Pays-Bas* 1991; **110**: 63–74.
24. Kaptein B, Boesten WHJ, Broxterman QB, Peters PJH, Schoemaker HE, Kamphuis J. Enzymatic resolution of α,α-disubstituted α-amino acid esters and amides. *Tetrahedron: Asymm.* 1993; **4**: 1113–1116.
25. Brückner H, Langer M. Gas chromatography separation of diasteroisomeric esters of α-alkyl-α-amino acids on dimethylpolysiloxane. *J. Chromatogr.* 1990; **521**: 109–121.
26. Khalil EM, Subasinghe NL, Johnson RL. An efficient and high yield method for the *N-tert*-butoxycarbonyl protection of sterically hindered amino acids. *Tetrahedron Lett.* 1996; **37**: 3441–3444.
27. Roeske R. Preparation of *t*-butyl esters of free amino acids. *J. Org. Chem.* 1963; **28**: 1251–1253.
28. Carpino LA. 1-Hydroxy-7-azabenzotriazole. An efficient peptide coupling reagent. *J. Am. Chem. Soc.* 1993; **115**: 4397–4398.
29. Bonora GM, Mapelli C, Toniolo C, Wilkening RR, Stevens ES. Conformational analysis of linear peptides: 5. Spectroscopic characterization of β-turns in Aib-containing oligopeptides in chloroform. *Int. J. Biol. Macromol.* 1984; **6**: 179–188.
30. Kopple KD, Ohnishi M. Conformations of cyclic peptides. IV. Nuclear magnetic resonance studies of cyclopentaglycyl-L-leucyl and cyclodiglycyl-L-histidyldiglycyl-L-tyrosyl. *Biochemistry* 1969; **8**: 4087–4095.
31. Martin D, Hauthal HG. *Dimethyl Sulphoxide*. Van Nostrand-Reinhold: Wokingham, UK, 1975.
32. Kopple KD, Shamper TJ. Proton magnetic resonance line broadening produced by association with a nitroxide radical in studies of amide and peptide conformation. *J. Am. Chem. Soc.* 1972; **94**: 3644–3646.
33. Toniolo C, Polese A, Formaggio F, Crisma M, Kamphuis J. Circular dichroism spectrum of a peptide 3_{10}-helix. *J. Am. Chem. Soc.* 1996; **118**: 2744–2745.

